

STUDIES OF THE *PINUS RIGIDA*-*SEROTINA* COMPLEX I. A STUDY OF GEOGRAPHIC VARIATION¹

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ABSTRACT

Pinus rigida and *P. serotina*, two closely related taxa which inhabit the eastern United States, have been described historically as varieties and subspecies of *P. rigida* and as separate species. These taxa are allopatric except for a narrow contact zone coincident with the Delmarva Peninsula and southeastern New Jersey. In this contact zone intermediate specimens have been encountered, and these have been interpreted both as belonging to clinally intermediate populations of a single continuum and as natural hybrids between two sympatric taxa. The present study was undertaken to provide additional information about the relationship of *P. rigida* and *P. serotina* and the composition of populations in the contact zone.

A number of populations were sampled from the natural ranges of the two taxa and from the transition zone. Bud and seed characteristics, along with cone length, were found to be quite similar in the two taxa. Other features, such as needle length, peduncle length, cone diameter, and serotiny differed. All of these characters varied clinally within each taxon, and these clinal patterns converged toward the transition zone. The natural populations of this zone were shown to possess characteristics of a single taxon that were morphologically intermediate between nearby populations of the two taxa. They were interpreted as being intermediate populations of a single geographically variable complex. Evidence was detected of a local steepening of the morphological gradation through the transition zone, and this evidence was interpreted as supporting a system of secondary intergradation, rather than primary radiation.

The authors follow Clausen (1939) in denoting the taxa as subspecies: *P. rigida* subsp. *rigida* and *P. rigida* subsp. *serotina*, because they fail to maintain their separate morphological and reproductive integrities in the contact zone, where they coalesce into a single entity.

Pitch pine (*Pinus rigida* Mill.) and pond pine (*P. serotina* Michx.) are two closely related taxa of the genus *Pinus* L. subsection *Australes* Loud. which have long been the objects of taxonomic uncertainty. They have been described as distinct species (Shaw, 1914; Coker & Totten, 1945; Little, 1953), as varieties of *P. rigida* (Louden, 1838; Sargent, 1926; Sudworth, 1927; Harrar & Harrar, 1962), and as subspecies of *P. rigida* (Clausen, 1939).

The most commonly cited diagnostic features for these taxa are needle length, cone shape, size and persistence of prickles, and degree of cone serotiny. Clausen (1939) described a cline in needle length which extended from pitch pine in Massachusetts to pond pine in Florida and which passed through a limited contact zone coincident with the Delmarva Peninsula and southeastern New Jersey. He also observed that the size and persistence of prickles were quite variable within each taxon, and that serotiny was not entirely reliable as a

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diagnostic trait, being present in both taxa in areas with a prolonged fire history. Although Clausen interpreted the sympatric populations as clinal intermediates, Little *et al.* (1967) considered the intermediate specimens in the contact zone to be the result of natural hybridization between distinct taxa.

Interpretation of preliminary field observations of the present study agreed with the views of Clausen (1939), but the variation patterns encountered suggested that environmental factors other than latitude might play an important role in the variation observed. The presence of loblolly pine (*P. taeda* L.) in the contact zone complicated the issue, because natural hybridization of this species with *P. rigida* and *P. serotina*, as described by Little *et al.* (1967), was apparent. In addition, the possibility of hybridization with shortleaf pine (*P. echinata* Mill.) could not be ruled out. It became apparent, therefore, that a thorough field study would be necessary to resolve the situation. The present paper deals only with *P. rigida* and *P. serotina*, while the relationships and hybridization of these two taxa with *P. taeda* and *P. echinata* are covered in a subsequent paper (Smouse & Saylor, 1973).

The objectives of this study were: (1) to describe the variation which exists within and among populations of pond and pitch pines, exclusive of the transition zone, (2) to determine to what extent such variation is associated with major geographical and environmental factors, and (3) to determine whether the transitional populations consist of both taxa, both taxa and natural hybrids, or populations of a single taxon that follow a genetic and/or environmental continuum.

SAMPLING PROCEDURE

A number of populations within and near the natural ranges of *Pinus rigida* and *P. serotina* and within the transitional zone between these taxa were chosen for study. Samples were obtained from a wide variety of geographical and environmental situations (Fig. 1). In and near the transition zone, populations were chosen for sympatry with *P. taeda* and/or *P. echinata*, but more distant population samples were only of a single taxon.

A sample of 15 trees was obtained from each of the taxa present at each location, if that many were available. Natural hybrids were also sampled wherever encountered. Details of the sampling procedures are given by Smouse (1970). Any tree suggestive of a hybrid combination involving either *Pinus taeda* or *P. echinata* was removed to avoid bias in the interpretation of the *P. rigida*-*P. serotina* analysis.

Trees were sampled for mature and yearling cones, foliage, and oleoresin. Collections for all but the oleoresin were restricted to the upper third of the crown whenever possible.

Within tree sampling involved 10 needle fascicles and 5 observations each for mature and yearling cones, dormant buds, and seeds. It was not always possible to obtain the desired numbers of observations for mature and yearling cones or dormant buds, however, so unweighted tree means were used for all analyses. Nine morphological characters were finally chosen for examination. Only trees with a complete set of nine character values were used in analysis.

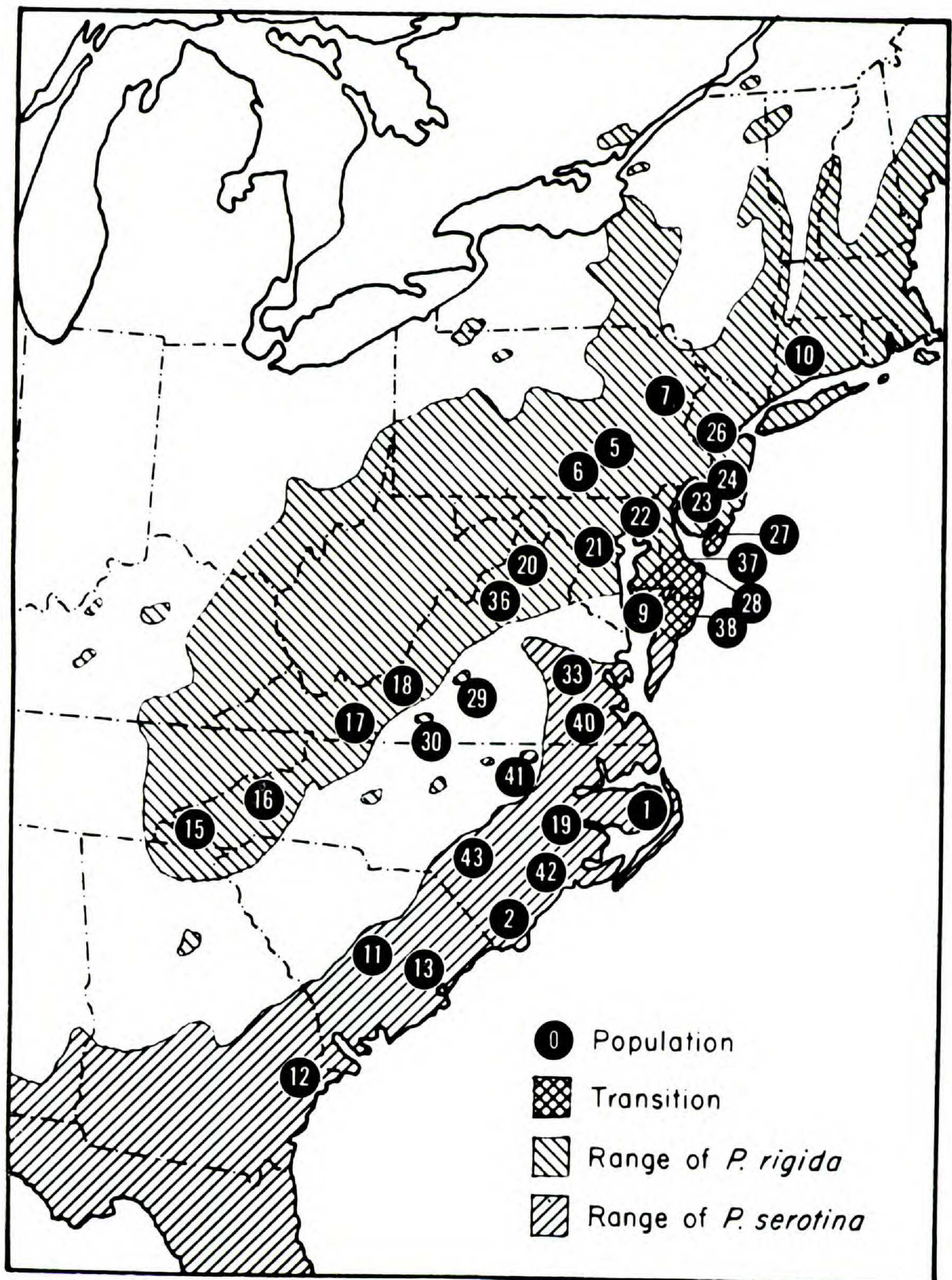


FIGURE 1. Range map of *Pinus rigida* and *P. serotina*, showing locations of sample populations.

ANALYSIS AND RESULTS

Statistical Strategy: To achieve all three objectives with any precision it was necessary to develop a unified statistical approach. Two different interpretations of the transitional populations were posed as alternative hypotheses. The first hypothesis was that each of the transitional populations represented a clinally intermediate population between the two extremes of a genetic continuum. The second hypothesis was that each of these populations consisted of two taxa

(pitch and pond pines), possibly with hybrids. To distinguish between these alternatives it was necessary to: (1) detect and measure the variation within each taxon, while ignoring the transitional populations, (2) construct an abstract picture (standard image) of each of the taxa at each location of the transitional populations, and (3) compare each transitional specimen with the images of the two taxa.

If the transitional specimens consisted of a mixture of both taxa (with or without hybrids), their distribution should be distinctly bimodal, and the variance within the mixture should greatly exceed that within either taxonomic standard. If the transitional specimens consisted of a single taxon, then the distribution should be unimodal, and the variation within the transitional type should be comparable to that within each of the taxonomic standards.

Geographic Variation: Mean values for each of the morphological characters differed among locations; in addition, there was considerable variation within populations (Table 1). Cone serotiny was difficult to quantify, so a subjective scoring procedure was devised; this was based upon the maximum number of years that cones remained closed on a given tree (Table 2).

Differences between the taxa were small (relative to the variation within taxa) for fascicle sheath diameter, cone length, seed thickness, and seed wing length. Differences for needle length, peduncle length, cone width, cone shape index, and serotiny score were more pronounced, although the ranges of variation within the taxa overlapped somewhat. An examination of Tables 1 and 2, along with Figure 1, reveals a tendency for decreasing needle length and peduncle length with increasing latitude within both taxa. The same characters increase in value with elevation in *Pinus rigida*, but since elevation and latitude are inversely correlated for *P. rigida*, it is not possible to clearly separate the elevational from the latitudinal associations. Cone width tends to decrease toward the transition zone in both taxa, while cone shape index increases toward the transition zone.

Precise measurement of clinal variation is a problem in regression analysis. The use of multiple regression for the description of geographic variation in a single character is a well known technique. Details of the type of multivariate regression analysis employed for the description of geographic variation in the present study are given by Smouse (1970).

The method results in a set of regression equations which describe variation in each of the characters as a linear function of four environmental variables: logarithm of latitude (X-1); logarithm of elevation (X-2); potential solar radiation (X-3)⁴; and a measure of site productivity (X-4). These environmental measures were intended only as rough indices for the various habitats. To the extent that they described the totality of associated environmental features of importance (e.g. rainfall and temperature regimes, photoperiodic phenomena, and general site fertility), they were expected to be useful for interpretation of the pattern of morphological variation encountered in the field.

⁴ Defined as total potential incident solar beam irradiation per annum, measured in hundreds of Langleys (kg-cal/cm²), and tabulated by Frank and Lee (1966).

TABLE 1. Population means and variances for eight taxonomic traits of *Pinus rigida*, *P. serotina*, and transitional populations.

Population (number)	Trait ^a							
	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅	Y ₆	Y ₇	Y ₈
<i>Pinus rigida</i>								
(15)	135(179)	231(738)	8(1)	61(62)	36(3)	25(4)	197(135)	170(367)
(16)	119(363)	222(281)	8(8)	59(65)	36(11)	22(8)	184(211)	164(176)
(17)	124(339)	255(1014)	6(6)	55(38)	35(6)	20(7)	180(425)	161(245)
(30)	134(303)	239(807)	9(14)	62(62)	39(15)	23(11)	178(198)	158(205)
(29)	105(0)	247(924)	9(0)	69(12)	41(1)	25(0)	193(9)	168(60)
(18)	113(74)	230(304)	8(3)	56(33)	34(6)	22(8)	173(462)	168(234)
(36)	135(89)	249(688)	10(6)	55(62)	35(10)	21(6)	173(128)	156(120)
(20)	132(29)	243(187)	7(4)	56(48)	36(15)	21(9)	188(129)	158(312)
(21)	129(431)	239(187)	8(7)	57(30)	35(9)	22(11)	176(211)	165(373)
(23)	103(131)	216(253)	3(1)	58(25)	35(7)	22(8)	158(209)	165(120)
(22)	116(140)	236(407)	7(3)	56(23)	33(5)	21(4)	162(181)	169(177)
(06)	80(42)	222(279)	5(4)	53(41)	33(7)	20(5)	175(131)	162(84)
(24)	111(92)	217(278)	4(4)	54(25)	34(11)	22(3)	155(145)	163(200)
(26)	119(222)	233(374)	7(13)	62(48)	37(15)	23(13)	167(149)	168(70)
(05)	111(120)	227(140)	5(2)	55(38)	33(8)	19(6)	172(120)	167(276)
(07)	105(173)	219(281)	4(3)	51(62)	31(15)	18(3)	176(253)	162(124)
(10)	100(315)	234(131)	5(1)	49(123)	31(21)	20(6)	174(105)	154(263)
Transitional Populations								
(38)	148(533)	237(268)	8(5)	56(24)	34(6)	21(7)	153(163)	163(200)
(09)	156(-)	259(-)	13(-)	64(-)	36(-)	25(-)	155(-)	175(-)
(37)	151(383)	239(464)	8(6)	61(41)	38(12)	22(3)	170(211)	163(173)
(28)	133(125)	250(160)	9(4)	67(35)	39(13)	23(5)	177(291)	170(128)
(27)	129(181)	230(270)	5(8)	66(31)	40(14)	24(5)	165(169)	168(215)

^a The taxonomic traits are:
Y₁ = Needle length (mm)
Y₂ = Sheath diameter (.01 mm)
Y₃ = Peduncle length (mm)
Y₄ = Cone length (mm)
Y₅ = Cone width (mm)
Y₆ = Seed wing length (mm)
Y₇ = Seed thickness (.01 mm)
Y₈ = Cone length/cone width
A(B): A = Population mean; B = Population variance

TABLE 1. (Continued)

Population (number)	Trait ^a							
	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅	Y ₆	Y ₇	Y ₈
<i>Pinus serotina</i>								
(12)	211(226)	270(485)	13(9)	58(101)	38(10)	20(12)	161(155)	154(306)
(13)	198(459)	240(186)	11(8)	58(37)	39(16)	21(8)	164(52)	147(117)
(11)	211(509)	249(358)	12(9)	60(25)	40(14)	24(6)	167(115)	148(63)
(02)	170(294)	244(306)	11(6)	51(11)	34(5)	29(2)	155(115)	149(153)
(42)	189(623)	246(761)	12(13)	56(19)	41(12)	22(7)	158(262)	137(39)
(19)	169(562)	239(394)	10(16)	61(68)	40(25)	22(11)	161(272)	149(130)
(43)	185(371)	251(373)	11(11)	66(55)	44(24)	25(14)	172(255)	139(160)
(01)	190(233)	251(689)	11(11)	62(33)	43(12)	21(5)	168(218)	143(135)
(41)	154(662)	231(961)	9(5)	67(7)	48(16)	27(1)	181(457)	139(91)
(40)	183(239)	256(143)	14(11)	58(16)	40(9)	22(7)	171(318)	144(75)
(33)	167(492)	248(618)	10(6)	60(99)	37(24)	22(15)	158(73)	160(196)

TABLE 2. Degree of cone serotiny (populations arranged by increasing latitude).

Population	Number of Trees Score ^a			Average value	Population	Number of trees Score ^a			Average value
	1	3	5			1	3	5	
<i>Pinus rigida</i>					<i>Pinus serotina</i>				
(15)	9	0	0	1.00	(12)	0	2	7	4.56
(16)	10	0	0	1.00	(13)	0	9	4	3.62
(17)	12	0	0	1.00	(11)	0	6	6	4.00
(30)	12	0	0	1.00	(02)	0	0	9	5.00
(29)	2	0	0	1.00	(42)	0	0	11	5.00
(18)	9	0	0	1.00	(19)	0	0	12	5.00
(36)	12	0	2	1.57	(43)	6	2	4	2.67
(20)	10	0	0	1.00	(01)	0	0	16	5.00
(21)	12	0	0	1.00	(41)	1	0	2	3.67
(23)	14	0	0	1.00	(40)	7	4	4	2.60
(22)	18	0	0	1.00	(33)	4	4	8	3.50
(06)	12	0	0	1.00	Transitional Populations				
(24)	4	5	2	2.64	(38)	15	0	1	1.27
(26)	6	6	1	2.23	(09)	1	0	0	1.00
(05)	11	0	0	1.00	(37)	15	1	0	1.12
(07)	10	0	0	1.00	(28)	16	6	3	1.96
(10)	4	0	0	1.00	(27)	15	4	4	2.17

^a Scoring system:
1 = Not serotinous.
3 = Maximum serotiny no more than one year.
5 = Maximum serotiny more than one year.

Two of the nine characters were removed from the analyses, because they were statistically objectionable. The serotiny index was not a continuous variable and was too crudely scored to justify its inclusion in a sensitive statistical analysis. Cone length-width ratio was also removed for reasons given below. The statistical analyses employed in the study assumed a normal distribution for each character. Both cone lengths and cone widths were symmetrically distributed and were close to being normally distributed. These characters were strongly correlated, which accounts for the utility of the shape index. The ratios of two normally distributed variables are not normally distributed, however, and when a high correlation exists between the components, the distribution is not necessarily even symmetrical. In the present instance symmetry was considered the more desirable property, because of the necessity of projecting taxa to new locations. A careful preliminary check also indicated that the ratio provided no information that could not be obtained from cone length and width jointly. Consequently, the shape index was dropped.

Using multivariate test procedures, it was determined that the association between morphological and environmental variation was very highly significant within each of the two taxa. However, the multivariate test is for composite association and must be broken into the univariate (single character) components for detailed analyses. This was easily accomplished, since the regression equation for each single character was the same as that which would have resulted from a univariate multiple regression analysis. Each character was separately subjected to an analysis of covariance, as shown in Table 3. No attempt was made

TABLE 3. Analysis of covariance form.

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F tests
<i>Pinus rigida</i>				
Among populations	16	$S_1 = S_2 + S_3$	$M_1 = S_1/16$	$M_1/M_4 = F_{16,166}$
Regression	4	S_2	$M_2 = S_2/4$	$M_2/M_4 = F_{4,166}$
Lack of fit	12	S_3	$M_3 = S_3/12$	$M_3/M_4 = F_{12,166}$
Within populations	166	S_4	$M_4 = S_4/166$	
<i>Pinus serotina</i>				
Among populations	10	$S_1 = S_2 + S_3$	$M_1 = S_1/10$	$M_1/M_4 = F_{10,117}$
Regression	4	S_2	$M_2 = S_2/4$	$M_2/M_4 = F_{4,117}$
Lack of fit	6	S_3	$M_3 = S_3/6$	$M_3/M_4 = F_{6,117}$
Within populations	117	S_4	$M_4 = S_4/117$	

to separate the contributions of the four environmental factors, since these factors were rather highly correlated. The lack of fit term, a measure of the failure to describe completely the morphological differences among populations by the four environmental indices, was estimated separately from the within population variation.

Population differences were large for all characters in *Pinus rigida*, and the environmental covariates were collectively effective in describing this variation for all characters except cone length (Table 4). The lack of fit terms were also generally large, indicating the presence of still “unexplained” geographical variation.

Population differences were evident within *Pinus serotina* for needle length, cone length, closed cone diameter, and seed wing length. The regression analyses were quite effective for needle length and cone diameter but were less so for cone length and seed wing length. Significant lack of fit was likewise evident for needle length and cone diameter. Although the lack of fit for all

TABLE 4. F-Values for tests of differences among populations, for utility of regression and for lack of fit.

Source of variation	Character ^a						
	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅	Y ₆	Y ₇
<i>Pinus rigida</i>							
Among populations	13.08*	3.68*	7.27*	3.08*	4.90*	3.89*	6.18*
Regression	26.09*	6.09*	12.76*	4.14	8.32*	9.36*	18.23*
Lack of fit	8.87*	2.87*	5.44*	2.72*	3.75*	2.07	2.16
<i>Pinus serotina</i>							
Among populations	7.51*	1.88	2.23	3.64*	7.35*	3.25*	2.43
Regression	9.50*	.28	.93	4.46	7.52*	5.35	.91
Lack of fit	6.18*	2.94	3.09	3.10	7.24*	1.85	3.43

^a The characters are:
Y₁ = Needle length (mm)
Y₂ = Fascicle sheath diameter (.01 mm)
Y₃ = Peduncle length (mm)
Y₄ = Cone length (mm)
Y₅ = Closed cone diameter (mm)
Y₆ = Seed wing length (mm)
Y₇ = Seed thickness (.01 mm)
* = Significant at the 5% level

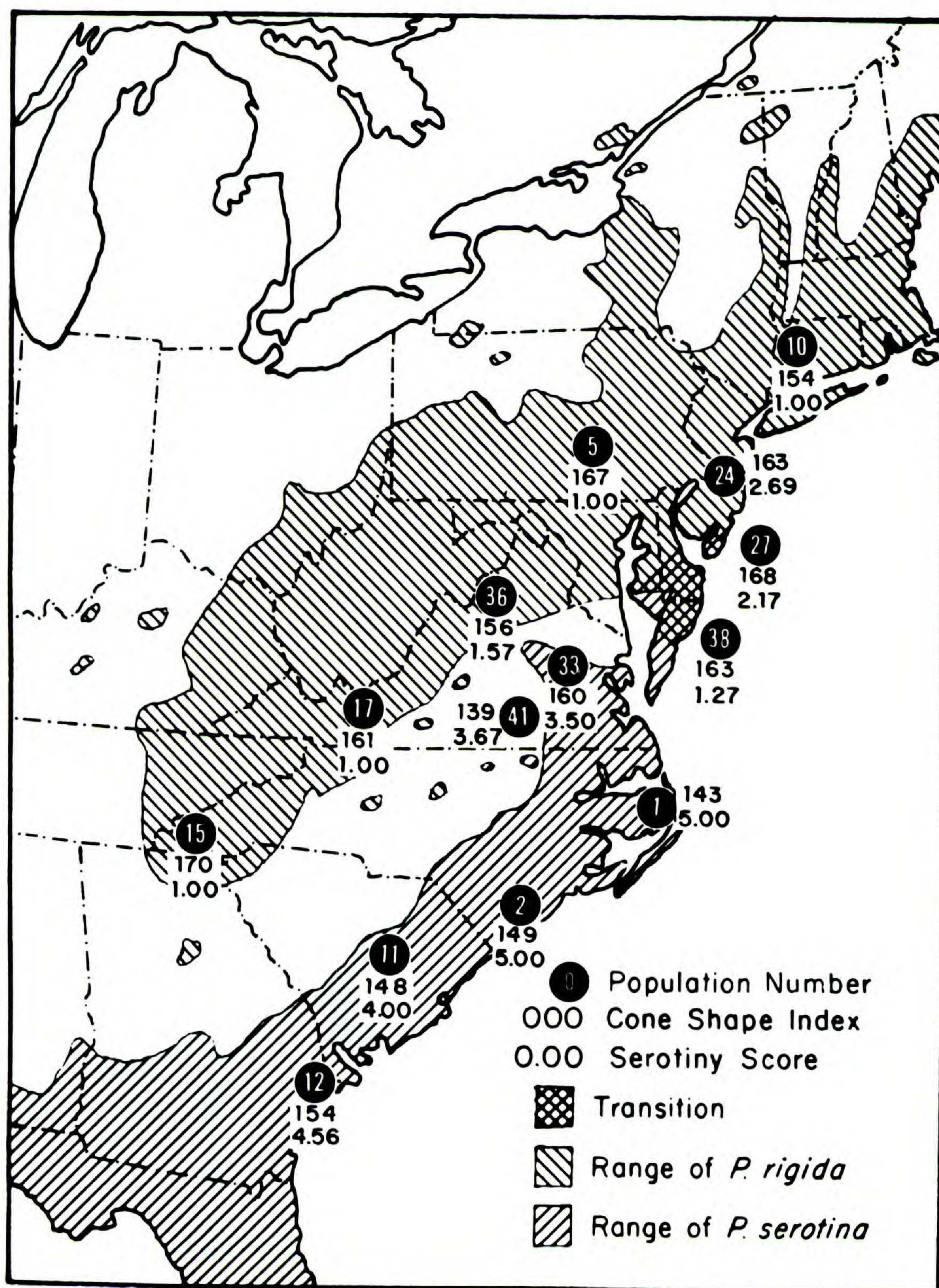


FIGURE 2. Range map of *Pinus rigida* and *P. serotina*, showing cone shape index and serotiny score for selected populations.

characters in both taxa was an appreciable portion of the total variation among populations, the regression mean squares (and F values) were generally larger than those for lack of fit, and the attempt to associate morphological and environmental variation was judged to be a general success.

Serotiny score and cone shape index were not subjected to statistical analysis, but a general picture of their respective variation patterns can be obtained from Figure 2. Serotiny score shows a clear geographic pattern. The closed cone

habit in *Pinus rigida* is lacking except in two areas represented by populations 24 and 36; both of these are near the range of *P. serotina*. Serotiny varies in *P. serotina*, decreasing as one moves northward and up onto the piedmont. It is interesting that the serotiny scores of populations 27 and 38 are less than that of population 24. Ledig⁵ (personal communication) has shown that serotiny in *P. rigida* is most prevalent in the Pine Barrens of New Jersey, and decreases in all directions away from that area. Whether this pattern represents past influence from *P. serotina* or locally severe selective pressures, it is clear that the transitional populations are intermediate between the two taxa for this character. Cone shape index values are not intermediate for the transitional populations, being larger than is usual within either taxon. This seems to be the result of tendencies for increasing cone length and decreasing cone diameter near the transition zone within both taxa.

Several other characters not included in the analyses warrant some attention. The size and shape of cone prickles were not scored in this study, because preliminary observations indicated that the pattern of variation paralleled that of serotiny, *i.e.* the greater the degree of serotiny, the less evident the prickles. Specimens of yearling cones and dormant buds were not available in sufficient quantities to include in the analyses, but it was clear that conelets and buds were almost indistinguishable for the two taxa.

Limited data have been published on the oleoresin composition of these two taxa, which would indicate that the taxa differ qualitatively in composition of the monoterpene fraction of the oleoresin. *Pinus rigida* was found on at least one occasion (Hamrick, personal communication)⁶ to contain appreciable quantities of β -Phellandrene, while *P. serotina* has been characterized as having large quantities of *l*-Limonene (Mirov, 1961). Our own analyses are not complete, but preliminary observations indicate that separation of these two taxa on only qualitative differences may not be possible. About half of the *P. rigida* trees examined to date possessed neither β -Phellandrene nor *l*-Limonene in appreciable quantities, whereas over half of the *P. serotina* trees examined were found to possess β -Phellandrene rather than *l*-Limonene.

In view of the claim that these two taxa grade together clinally, it is of considerable interest to obtain an overview of the pattern of the variation. A separate description of all characteristics is precluded by limitations of space. The general pattern of clinal variation found within each taxon indicated that the two taxa are much more similar near the transition zone than farther away. The overall morphological pattern was rather striking, as can be seen in Figure 3, which was constructed as follows. A discriminant function was computed from the taxon means and the residual covariance matrices. A com-

⁵ Dr. F. Thomas Ledig, School of Forestry, Yale University, New Haven, Connecticut.

⁶ Unpublished internal report by J. L. Hamrick, Institute of Forest Genetics, Placerville, California.

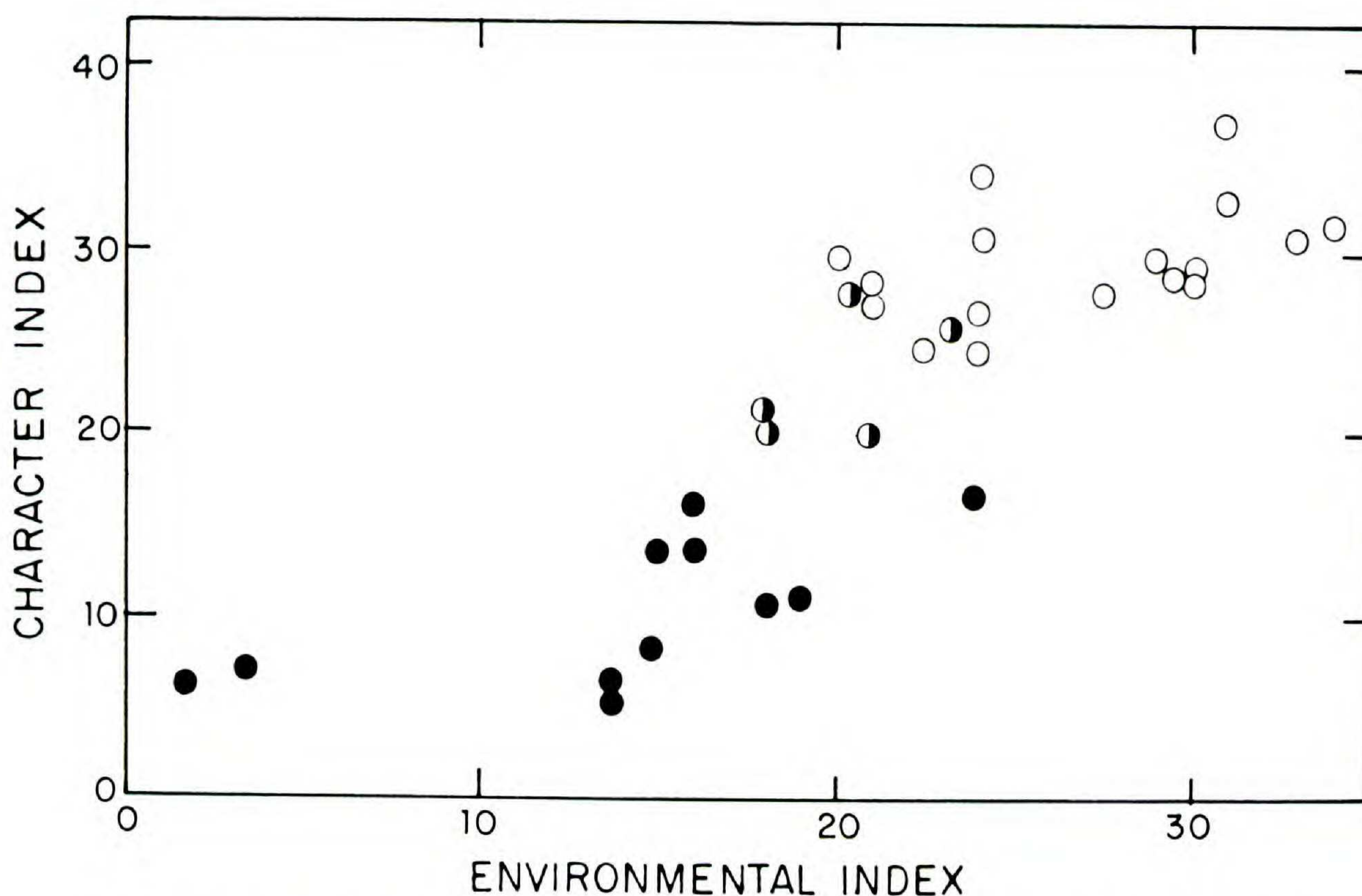


FIGURE 3. Variation of a morphological character index among populations of *P. rigida*, open circle; *P. serotina*, closed circle; and the transitional type, half-closed circle, in relation to an environmental index.

combination character index was then obtained by scoring each tree according to equation (1):

$$(1) \quad Y^* = a_1Y_1 + a_2Y_2 + \dots + a_7Y_7,$$

where the a 's are the character weights derived from the discriminant analysis. Next, an average regression equation was obtained for each character by averaging the separate equations for the two taxa. A combination environmental index was then constructed by subjecting the regression equations to the same set of weights as the morphological characters. The character index was then plotted against the environmental index (Fig. 3) in an effort to portray graphically the clinal patterns within and between these taxa. The picture is one of two clinally varying taxa connected by a series of intermediate or transitional populations. It is clear that there is no discontinuity, but the gradient does steepen in the transition area.

Although Figure 3 suggests that the transitional populations may consist of a single clinally intermediate type, it is not in itself conclusive. The average of two distinct taxa at these locations might well yield a similar result. In addition, Figure 3 shows an average environmental trend for the two taxa and an average morphological appearance. To provide a more rigorous evaluation, a separate comparison was made of each transitional specimen with each of the taxa as these taxa would appear if they actually existed in the transitional area.

Construction of Taxonomic Images: To determine the likely appearance of each taxon at each of the transitional locations (without reference to the speci-

TABLE 5. Means and variance of projected *Pinus rigida* and *P. serotina* and of the transitional populations.

Population	Taxon	Character ^a						
		Y ₁	Y ₂	Y ₃	Y ₄	Y ₅	Y ₆	Y ₇
—	<i>P. rigida</i> (Unadjusted species means)	117(286)	233(454)	7(7)	57(49)	35(11)	21(7)	174(214)
(09)	<i>P. rigida</i> (Adjusted Transitional)	140(313)	239(498)	8(8)	61(54)	38(12)	25(8)	160(234)
		156(—)	259(—)	13(—)	64(—)	36(—)	25(—)	155(—)
(27)	<i>P. serotina</i> (Adjusted)	161(579)	241(557)	9(13)	68(58)	46(23)	24(10)	169(243)
	<i>P. rigida</i> (Adjusted Transitional)	127(300)	232(478)	7(7)	59(52)	36(12)	24(8)	159(225)
		129(181)	230(270)	5(8)	66(31)	40(14)	24(5)	165(169)
(28)	<i>P. serotina</i> (Adjusted)	142(722)	230(695)	7(16)	77(72)	55(29)	27(13)	176(303)
	<i>P. rigida</i> (Adjusted Transitional)	124(321)	239(511)	6(8)	61(55)	37(13)	25(8)	150(241)
		133(125)	250(160)	9(4)	67(35)	39(13)	23(5)	177(291)
(37)	<i>P. serotina</i> (Adjusted)	159(554)	239(532)	10(12)	62(56)	43(22)	22(10)	166(232)
	<i>P. rigida</i> (Adjusted Transitional)	141(307)	239(488)	8(7)	59(53)	37(12)	24(8)	168(230)
		151(383)	239(464)	8(6)	61(41)	38(12)	22(3)	170(211)
(38)	<i>P. serotina</i> (Adjusted)	168(544)	250(532)	11(12)	63(55)	41(22)	23(10)	168(228)
	<i>P. rigida</i> (Adjusted Transitional)	139(296)	239(471)	7(7)	59(51)	37(12)	24(8)	166(222)
		148(533)	237(268)	8(5)	56(24)	34(6)	21(7)	153(163)
—	<i>P. serotina</i> (Adjusted)	167(529)	247(509)	11(11)	62(53)	41(21)	23(10)	169(222)
	<i>P. serotina</i> (Unadjusted species means)	187(507)	248(487)	11(11)	60(51)	41(20)	23(9)	165(212)

^a The characters are:
Y₁ = Needle length (mm)
Y₂ = Sheath diameter (.01 mm)
Y₃ = Peduncle length (mm)
Y₄ = Cone length (mm)
Y₅ = Cone width (mm)
Y₆ = Seed wing length (mm)
Y₇ = Seed thickness (.01 mm)
A(B): A = Mean; (B) = Variance.

mens actually obtained at these locations), an abstract standard was constructed for each taxon by extrapolative regression procedures. Each standard consisted of an estimated array of seven character means and a corresponding covariance matrix. The details of this procedure are to be found in Smouse (1970), but it is worth noting that the covariance matrix in question was the residual covariance matrix. This matrix included both the within population variation and the lack of fit variation, and served as a conservative estimate of all variation within a taxon (after removal of the clinal variation). The estimated means and variances of the seven characters are listed for each taxonomic standard and each transitional population in Table 5. All further references to the two taxa at the transitional locations should be understood to refer to the abstractions constructed as indicated above.

It can be seen from these data that for transitional locations, except location 27, the means of the estimated standards of the two taxa are more similar than the comparable means for the unadjusted averages of these same taxa. This general morphological convergence of the taxa in the transitional locations is not surprising in view of the patterns exhibited in Figure 3. This indicates the necessity of allowing for geographically associated morphological variation when contrasting the two taxa.

In spite of the similarity of the two taxonomic standards, however, the character means of the transitional populations are not all intermediate between the appropriate mean values for the taxa. Two factors should be considered. First, the projections are subject to estimation errors. This is particularly true since the responses of the taxa to environmental variation at the edges of their respective ranges may not be strictly linear, as are the projections. Second, most of the actual values obtained for the transitional locations deviate from the average of the two images no more than the means of environmentally similar populations within each taxon deviate from each other. Each of the transitional population mean values is well within the range of variation of either taxonomic standard appropriate for the location in question. Thus, it appears that each of the transitional populations represents some sort of average between the two taxa.

The projected means of *Pinus serotina* at location 27 appear to be suspect. Cone length (Y_4) and cone width (Y_5) are particularly conspicuous by their deviations from other *P. serotina* mean values. Such deviations might be a reflection of two situations. The location in question is well outside the sampled range of *P. serotina*, and it is possible the values presented are a good reflection of what *P. serotina* really would look like if it were growing at that location. Alternatively, it is possible that estimation errors are involved in the considerable environmental extrapolation necessary to construct the standard. It is not possible to determine which condition is the more likely cause of these extreme values. In any event, the standard is the best available from the data at hand.

Discriminant Analysis: The next step in the analysis was to construct a discriminant function of the form shown in equation (1) for each of the transitional locations. Since the means and covariance matrices were different at each location, the resulting functions were also different. By arbitrarily scaling the

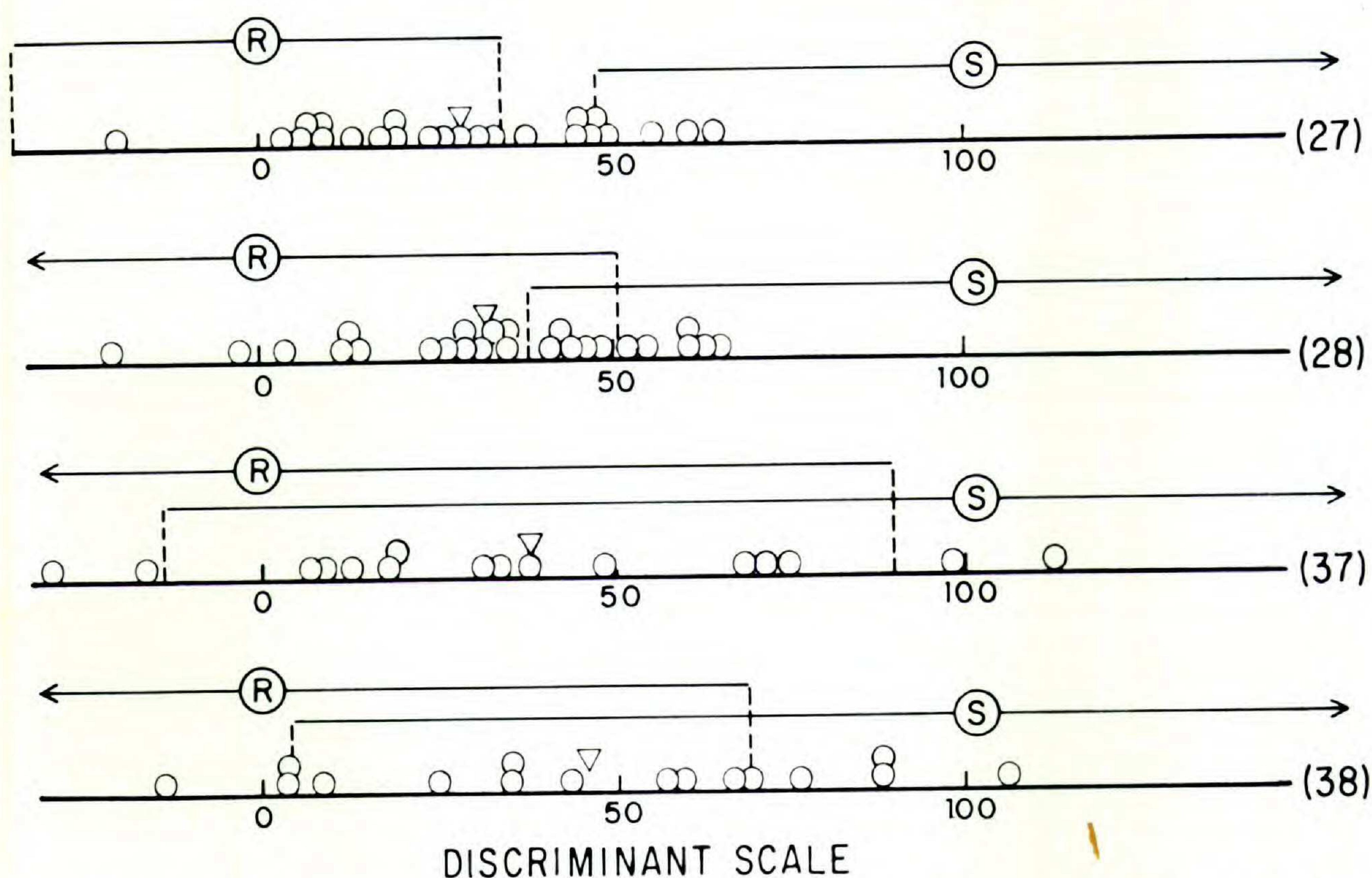


FIGURE 4. Discriminant analysis of transitional populations 27, 28, 37, and 38. Circled-R, mean and 95% confidence interval for *Pinus rigida*; circled-S, mean and 95% confidence interval for *P. serotina*; inverted triangle, mean of transitional population; open circle, discriminant score of a transitional specimen.

differences between the taxa to a constant, however, it was possible to compare the transitional locations (Fig. 4). Because population 09 was represented by only a single specimen, it is not shown. The theoretical 95% confidence intervals were computed by standard techniques.

Considerable overlap of the taxonomic standards at locations 37 and 38 is evident, even though the discriminant function maximizes the resolution of the taxa. The two taxonomic standards are more distinct at locations 27 and 28. There are too few specimens available at any of these locations to determine whether or not the variation was bimodal, but the variance within each of the transitional populations was quite comparable with that within either taxonomic standard. This observation supports the single taxon hypothesis.

Determination of whether one or a mixture of taxa exist in the transitional area can be approached in still another way. If both *Pinus rigida* and *P. serotina* were present in each of the transitional populations, then about half of the unidentified *P. rigida* specimens should have index values below the estimated mean of *P. rigida*, and about half above. A similar situation should also exist for the unidentified *P. serotina* individuals. This symmetry about the mean follows from the normality of the various characters, both individually and in linear combination. If we ignore the single specimen from population 09, we have only 8 out of 81 unidentified specimens falling either below the mean of *P. rigida* or above that of *P. serotina*. This would be a very unlikely result if two taxa were present.

It is possible that some of these 81 specimens are hybrids, and hybrids would

naturally fall between the taxa. Since this would lead to an overestimate of the number of species specimens between the taxonomic means, the hybrids should not be counted. Therefore, for the sake of argument, we might conservatively assume that no more than 40 of these 81 specimens represent one or the other taxon; for simplicity we might assume 20 specimens of each taxon. We should then expect about 20 values to fall between the means and about the same number to fall to one side or the other. The probability of obtaining as many as 32 between the means and as few as 8 to the outside is still negligibly small. There is no particular reason to assume equal numbers of the two taxa or that as many as 40 of the specimens belong to one or the other of the taxa, but the argument is sufficient to illustrate the point. It appears that the transitional populations represent clinally intermediate populations between the two extremes of a single genetic and/or environmental continuum.

DISCUSSION AND CONCLUSIONS

Serious discriminatory difficulties between the taxa actually arose in two zones. The first was the aforementioned transition zone. The second was a zone between populations 33 and 36, to the north and west of Richmond, Virginia. The two taxa are geographically close in this area (Fig. 1), and intermediate specimens were occasionally encountered. It may well be that this area either is or has been a second transition zone. This possibility is supported by the fact that population 33 is suggestive of *Pinus rigida* in many respects, while population 36 is equally suggestive of *P. serotina*.

The local steepness of the shift in these two zones could be used to support either of two hypotheses. The first is that the rapid morphological shift simply reflects a local steepening in an underlying selective gradient upon a series of populations which arose by adaptive radiation. The second is that this morphological gradation has resulted from secondary intergradation of two closely related taxa, rather than from primary radiation. No conclusive data are available to resolve this question, but the authors are more inclined to the second view.

Considerable evidence (Whitehead, 1965) is now available to indicate that the vegetation considerably to the south of the present transition zone was more boreal during maximum Wisconsin glaciation than at present, indicating a corresponding southward displacement of the more austral flora to which both of these taxa belong. The advance of glaciation provides a likely mechanism for inducing southward movement of a single taxon and geographic separation into two parts. Northward movement of both entities subsequent to glacial retreat could have resulted in sympatry in or near the present zone. The process of geographic isolation, adaptive radiation, and subsequent secondary intergradation need not have been completed in a single glacial cycle, there being four such periods in the Pleistocene.

The secondary intergradation hypothesis assumes that the two taxa are capable of hybridization. Artificial hybridization of these two taxa has demonstrated that crossability is very high (Smouse, 1970), but the lack of increased variation in the transition populations indicates that any excessive genetic segrega-

tion which might have followed such hybridization has been eliminated, probably by selection. As a result, a series of locally adapted, clinally intermediate populations now exist.

It is conceivable that hybridization of *Pinus rigida*, *P. serotina*, or their transitional type with one or more other taxa (*e.g.* *P. taeda*) may be the cause of some of the variation patterns encountered (*e.g.* for cone shape), particularly in the transition zone. While every effort was made to remove the influence of other taxa from the samples used here, it should be pointed out that the whole complex hybridizes naturally with *P. taeda*. Past influence of *P. taeda* on *P. serotina* was particularly evident in populations 41 and 43, but the infiltration appeared to be of such a long standing nature and so pervasive that removing this influence from *P. serotina* was not possible.

The primary objective of this study, specifically to describe the relationship of the transitional populations to both pond and pitch pines, was accomplished with new levels of precision. However, this in turn revived the problem related to classification of the various components of the *P. rigida-P. serotina* complex. Problems associated with determining taxonomic status have been well documented (*e.g.* Grant, 1963; Mettler & Gregg, 1969). These are related primarily to the question of how much morphological and/or reproductive differentiation is required before a pair of taxa should be considered separate species. Although criteria that can be objectively and practically applied are difficult to define, much of the controversy (*i.e.* taxonomic *vs.* biological species) can be avoided, if for a particular case, the criteria used for judgment are clearly stated.

As explained by Mettler and Gregg (1969), the term species is used in two ways: (1) as a taxonomic designation of organisms that are morphologically distinct, and (2) as a designation of entities which constitute reproductively isolated breeding populations. The first use is more convenient for cataloging, while the second is used more to reflect evolutionary relationships and restrictions of gene exchange. In the latter case, although ascertaining morphological differences is still quite necessary, the reproductive relationship among populations is considered more important than morphological differences between individuals. The test of sympatry, *i.e.* ability to maintain distinctness when coexisting in the same habitat, is of paramount importance. The types of classification dictated by these two points of view are not always compatible, and rather than adopt one or the other, the authors prefer to adopt a middle course.

It is common in plant taxonomy to employ varietal status for taxa which show minor morphological differences; generally, statistical differences exist in average appearance, but considerable overlap occurs among individual specimens. Reproductive barriers among varieties are generally lacking, and genetic exchange is quite easy, given the opportunity. Subspecies are usually more distinct morphologically, and reproductive barriers are greater than for varieties, although not absolute. Subspecies generally cannot withstand the test of sympatry, in spite of these barriers, and coalesce both genetically and morphologically where they come into contact. Species status is usually reserved for taxa which are clearly distinct morphologically and which are known or assumed

to be capable of withstanding the test of sympatry. Reproductive barriers need not be absolute, but the taxa should maintain their basic genetic and morphological integrity in sympatry, in spite of limited genetic exchange.

On the basis of the above criteria, the authors do not feel that separate specific status is warranted for pitch and pond pines for the following reasons. (1) In the only areas these two taxa are found together, they fail the test of sympatry. (2) As a complex, pitch and pond pines hybridize relatively freely with *Pinus taeda*, and with *P. echinata* to a slightly lesser degree. (3) All three entities (*P. rigida-serotina* complex, *P. taeda* and *P. echinata*) maintain their separate genetic and morphological integrities in sympatric populations in spite of the ability to hybridize.

Varietal status does not appear appropriate either. The morphological differences between *Pinus rigida* and *P. serotina*, taken as a whole, are not minor, in spite of clinal integradation through the transition zone. The steepness of the transitional gradient serves to connect but not erase these differences. The morphological differences are not as striking as those separating the whole complex from other species, notably *P. taeda* and *P. echinata* (Smouse & Saylor, 1972), but they are adequate to prevent serious ambiguity everywhere but in the vicinity of the transition zone. In addition, there is some evidence of partial reproductive isolation between the two taxa (Smouse, 1970).

The most meaningful classification in the authors viewpoint is to follow Clausen (1939) in denoting these two taxa as subspecies: *Pinus rigida* Mill. subsp. *rigida*, and *P. rigida* subsp. *serotina* (Michx.) Clausen.

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